STANDARDIZATION AND VALIDATION OF HINGWASHTAK CHURNA AND HINGWADI CHURNA

Research Protocol

For Registration in Ph.D. programme

To

JECRC UNIVERSITY, JAIPUR

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Dated……

CERTIFICATE

This is to certify that this Research Protocol entitled **"Standardization and validation of Hingwashtak churna and Hingwadi churna"** by **Khushaboo Kumari,** submitted in fulfilment of the requirement for registration in Ph.D. Program in DEPARTMENT OF BIOTECHNOLOGY under School of Science of JECRC University, Jaipur, during the academic year 2023 is a Bonafede record work carried out under our guidance and supervision.

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2.0 Introduction

Flora are the predominantly photosynthetic eukaryotes of the dominion plantae. Plant plays a crucial function in the renovation of existence on this planet. Medicinal vegetation may be defined because the plants that possess healing properties or exert useful pharmacological effect at the human or animal body. Medicinal vegetation has lengthy been utilized in conventional medicinal drug and worldwide ethnomedicine. Indian system of medicine has long use of herbs, metals, and minerals as the base in their medicinal formulations. For this reason, in current generation, all of us is looking closer to Ayurveda for efficacious remedy in small dose without any facet outcomes **(Dash et al., 2016).**

2.1 Glimpse of Ayurveda

Ayurveda is one of the traditional medicinal structures with a mounted history of many centuries. Moreover, called Ayurvedic medication, this historic Vedic knowledge is considered to be one of the oldest healing sciences and has survived until the gift era over many centuries of tradition. Originated in India hundreds of years in the past, Ayurveda is called the "mother of All recuperation"**.** Etymologically speaking, it is the combination of the Sanskrit phrases ayur (existence) and veda (technological knowledge or information), which means "the technological knowledge of existence," specializing in bringing harmony and stability in all areas of existence which include mind, frame and spirit **(Subramani et al., 2014).**

In Ayurveda, Panchamahabhutas or the five factors: Vayu (air), Teja (fire), Aap (water), Prithvi (earth) and Akasha (aether) are believed to accumulate the living microcosm (human beings) and the macrocosm (external universe). whilst mixed in pairs, the Panchamahabhutas shape Tridosha or the three humours namely Vata (liable for frame movement), Pitta (liable for bodily chemical reactions consisting of metabolism and temperature) and Kapha (liable for increase, protection, lubrication and sustain acne). Most of these present the charter or Prakriti of an individual, which determines the physical as well as intellectual function of human. The concept is that health is done when there's a balance between these three fundamental doshas, whereas imbalance causes diseases. Based on those Panchamahabhutas and Tridosha, the Prakriti of a

character is determined and a distinctive remedy plan may be prescribed in line with their specific constitution. **(Kshirsagar et al.,2011)**

The philosophy behind Ayurveda is preventing pointless suffering and dwelling a long healthy existence. In contrast to the allopathic drugs which uses especially synthetic chemical substances designed for unique target receptors and primarily give symptomatic comfort, Ayurveda entails the use of herbal manner including herbs, spices, minerals, exercise, meditation, yoga, intellectual hygiene, sounds, smells, weight loss plan and mechano-processes to put off the foundation purpose of the disease by way of restoring stability, on the same time create a healthful life-fashion to save you the reoccurrence of imbalance **(Parasuraman et al.,2014).**

2.2 The Antiquity of Ayurveda

In terms of literature, the fourth Veda written for the duration of Indian Civilization, Atharva-veda serves because the earliest genuine textual content discussing on the character of life, fitness and sickness, pathogenesis and standards of treatment. Here in Atharva-veda, the healing verses of Ayurveda can be normally discovered, wherein more than one hundred hymns had been noted because the healing procedures for diseases, together with fever, leprosy, consumption, coronary heart illnesses, wounds, headaches, parasites, eye and ear sicknesses, poisoning, rheumatism and epilepsy. The uniqueness of this historic scientific machine lies at the back of the good-sized sort of recuperation technique used: Charms, plant and animal juices, natural forces (solar and water) in addition to human contrivances **(Subramani et al., 2014).**

From the information in Atharva-veda, early texts of Ayurveda which include Charaka Samhita and Sushruta Samhita had been advanced. Even though the former focuses on the reasons of sicknesses and the charter of a person, the later emphasizes on Ayurvedic surgical procedure and the information of its strategies**.** The records of Ayurveda can be traced again to the period among the pre-vedic durations (4000 B. C.-1500 B. C.). Consistent with Ayurvedavatarana (the descent of Ayurveda), Lord Brahma, the Hindu God of advent exceeded on his “knowledge of existence” to Daksha Prajapati and Ashwins, eventually to Indra. This knowledge is then transferred to specific rishis (sages), in which these disciples of Ayurveda wrote exclusive treatises primarily

based on their interpretations. Here, each Bhardwaj and Dhanvantari received the understanding from Indra. They later advanced school of drugs and college of surgical operation respectively **(Dahanukar and Thatte.,1989).**

In Charaka Samhita, it was said that the Ayurvedic coaching is transferred by means of Indra to Bhardwaj, who in flip taught this to Atreya. The disciples of Atreya wrote their own samhitas, with Agnivesha Samhita being the one well-typical. it is then revised, edited and supplemented by using Charaka about 800 years later. On the opposite hand, Sushruta Samhita noted the transfer of information from Indra to Dhanvantari, together with Bhardwaj. The disciples in this faculty which includes Sushruta wrote Sushruta samhita, compiling Dhanvantari's teaching and his extra findings **(Subramani et al., 2014).**

2.3 Herbal Drugs

Because the prehistoric length, natural medicines have existed global-huge with long recorded history. They were used in historic Chinese, Greek, Egyptian and Indian medicinal drug for diverse therapies purposes; while the local American and African use herbs in their recuperation rituals as part of their lifestyle. The Indian Ayurvedic machine has included herbals as certainly one of its most effective recuperation components, which might be recorded inside the literature inclusive of Vedas and Samhitas **(Oreagba et al.,2011).**

Due to the provision of chemical analysis techniques in the early nineteenth century, scientists began to extract and regulate active compounds from the herbals, resulting in transition from raw herbs to artificial prescribed drugs. that is whilst using herbal drugs commenced to say no. Artificial prescribed drugs, but, are discovered out to be exceedingly greater highly-priced and produce severs unwanted facet-outcomes in spite of their strong pharmacological movement. as a result, human beings these days are moving back to natural drugs, that are originated from the nature and claim to be more secure **(Oreagba et al.,2011).**

2.4 Ayurvedic Herbals

Based on the material of origin, Ayurvedic drug treatments are divided into 3 training, specifically herbal, mineral and animal. Among this, herbal system has gained wonderful importance and rising worldwide interest lately. The world Health Organization (WHO) estimates that 80% of the word's inhabitants nonetheless depend especially on traditional medicines for their health care **(Kamboj.,2000).** The subcontinent of India is nicely-acknowledged to be one of the mega biodiversity facilities with approximately 45,000 plant species. **(Methew and Babu.,2011)** In India, about 15,000 medicinal plant life were recorded, in which the communities used 7,000-7,500 vegetation for curing distinctive illnesses. Ayurveda has approximately seven-hundred sort of flowers listed in its medicinal systems. **(Hasan et al.,2009)** the use of such herbals is cited in the ancient Ayurvedic literature along with Charaka Samhita and Sushruta Samhita.

The invention of herbals is in addition complemented with expertise on the method of isolation, purification, characterization of active elements and type of education. The time period "natural drug" determines the component/elements of a plant (leaves, vegetation, seeds roots, barks, stems and etc.) used for getting ready drugs. Each and each part of the herbs are fully utilized for the distinctive pharmacological action they will produce and made right into a variety of herbal preparations together with Kwatha (Decoction), Phanta (warm infusion), Hima (bloodless infusion), Arka (Liquid Extract), Churna (Powders), Guggul (Resins and balsams), Taila (Medicated oil) and so on. **(Frawley and Ranade.,2000)**

Therefore, ayurveda is consist of approx. hundreds of compound formulations, out of which I after huge review of literature search and selected two herbal formulations 1. Hingwadi 2. Hingwashtak as Hing the *Ferula assafoetida* is very important in Indian food and cusine. The typical smell of this herb exudate is very useful in digestion of food and appetite of stomach. Till now no such work on standardization and validation of these two herbal formulations has been performed so I selected the same for my research work.

Table 1; Ingredients and composition of Hingwashtak churna

S.No.	Common name	Botanical name	Use Part	Grams
1.	Sunthi	*Zingiber officinale*	Rhizome	3g
2.	Marica	*Piper nigrum*	Fruit	3g
3.	Pippali	*Piper longum*	Fruit	3g
4.	Ajamoda	*Trachyspermum ammi*	Fruit	3g
5.	Saindhava	*Sodium chloride*	Salt	3g
6.	Svetajiraka	*Cuminum cyminum Linn*	Fruit	3g
7.	Krsnajiraka	*Carum carvi*	Fruit	3g
8.	Hingu	*Ferula assafoetida*	Exd.	3g

Table 2; Ingredients and composition of Hingwadi churna

S.No.	Common Name	Botanical Name	Use Part	Portion
1	Hingu	*Ferula assafoetida*	Exd.	1 Part
2	Granthika	*Leonotis nepetifolia*	Root	1 Part
3	Dhanya	*Coriandrum sativum*	Fruit	1 Part
4	Jiraka	*Cuminum cyminum*	Fruit	1 Part
5	Vaca	*Acorus calamus*	Rhizome	1 part
6	Cavya	*Piper chaba*	Stem	1 part
7	Agni (citraka)	*Plumbago zeylanica*	Root	1 part
8	Patha	*Cyclea peltate*	Root	1 Part
9	Sati	*Curcuma zedoaria*	Rhizome	1 Part
10	Vrksamla	*Garcinia indica*	Fr. P	1 Part
11	Saindhava lavana	*Sodium chloride*	Salt	1 Part
12	Sauvarcala lavana	*Unaqua sodium chloride*	Salt	1 Part
13	Vida lavana	*Ammonium chloride*	Salt	1 Part
14	Sunthi	*Zingiber officinale*	Rhizome	1 Part
15	Marica	*Piper nigrum*	Fruit	1 Part
16	Pippali	*Piper longum*	Fruit	1 Part

17	Yavaksara	*Hordeum vulgare*		1 Part
18	Svarjikaksira	*Sodium carbonate*		1 Part
19	Dadima	*Punica granatum*	Dr. Sd.	1 Part
20	Pathya	*Terminalia chebula*	Fruit P	1 Part
21	Puskara(mula)	*Inula racemose*	Root	1 Part
22	Vetasamla	*Garcinia pedunculata*	Fruit	1 Part
23	Hapusa	*Juniperus communis Linn.*	Root	1 Part
24	Ajaji(svetajiraka)	*Cuminum cyminum*	Fruit	1 Part
25	Ardrakarasa	*Zingiber officinale*	Rhizome	Q.S.
26	Bijapurarasa	*Citrus medica*	Fruit	Q.S.

3.0 Review of Literature

- Conventional expertise on medicinal drug way the usage of flora available in the environment for the treatments of illnesses Curable properties of vegetation are evident from the times of our Vedas. Use of natural remedies is on the upward thrust in growing and advanced nations. Many plant extracts had been used as a source of medicinal retailers to treatment gastrointestinal disorders, breathing illnesses, cutaneous affection, parasitic protozoan illnesses, inflammatory approaches, urinary tract infections and so forth. Natural merchandise has served as a first-rate supply of medication for hundreds of years, and approximately 1/2 of the prescribed drugs in use now a days are derived from herbal products (**Deshmukh et al.,2018**).
- Plants are used in several systems of medicines like unani, Ayurveda and Homeopathy. Although it is expected that approximately 35,000 to 70,000 plants species are used as medicinal flora out of 422127 reported worldwide plant species, but only a limited variety of flora had been systematically investigated for their Bio-efficacy Activity**.** Different parts of medicinal plant life were used as medicine with the aid of the local traditional healers. A number of the distinctive plant parts, the leaves were maximum

regularly used for the remedy of diseases observed by means of entire plant elements, fruit, stem, root, stem and root bark, seed, flower and latex **(Deshmukh et al.,2018).**

- An important aspect of research in Ayurveda is Drug research which is very important for maintaining and supplementing drugs and their calibration. In Ayurveda, Churna is a first-class powder of a drug or capsules which is prepared through mixing smooth, finely powdered and sieved capsules. The term Churna can be carried out to the powder organized by way of a single drug or a mixture of greater tablets (**Aslam et al.,2016**).
- In keeping with WHO more than 80% of people inside the global depend on traditional medicinal process for their health care needs. In Indian and Chinese societies, herbal medicines are to be had for slight illnesses like cuts and burns to main illnesses like tumors and wounds in Indian and Chinese societies. It is quite apparent that natural preparations are evolved for a crucial event like infant birth within the society (**Parasuraman et al.,2014**).
- Herbal medicines have lots of advantages such as, herbal medicine have better patient tolerance as well as popularity. Improvements within the great, efficacy and protection of natural drug treatments with the improvement of technology and era. Lengthen and reputedly uneventful use of herbal drug treatments may provide testimony of them. Herbal drugs are very safe, efficacy and cheap in fee. Polyherbal drugs are now not dangerous and greater effective three an any synthetic drug (**Dasgupta andAmartya.,2012**).
- Globally, the early a part of the 20th century added an evolution inside the pharmaceutical industry. With progress of chemical techniques, crude tablets came to be changed through natural chemical capsules and developed nations witnessed a decline in popularity of medicinal plant therapy. But, in the course of latest beyond interest has swung once more and there's a resurgence of interest in have a look at and use of medicinal plant. Maximum of the traditional plant-based treatments are lower back in used and locate increasing software (**Amartya and Saroch.,2012**).
- Standardization of Ayurvedic formulations is a critical step for the establishment of a consistent biological interest, a consistent chemical profile, or actually an excellent assurance application for production and manufacturing of natural drugs. WHO

guidelines for the evaluation of the safety, efficacy and quality of herbal drugs are a prerequisite for international harmonization (**Nikam and Kareparamban.,2012**).

- Herbal drug era includes conversion of botanical substances into drug treatments where standardization and nice manage with right integration of current clinical strategies and conventional information is employed, and numerous drug delivery technologies used. The improvement of these traditional systems of drug treatments with the perspectives of safety, efficacy and first-class will assist no longer handiest to keep this traditional historical past but also to rationalize the usage of natural merchandise in the healthcare (**Choudhary and Singh.,2011**).
- Ethnobotany is the study of relationship between the plants and humans. Ethno "take a look at of humans" and Botany "look at of plant". Ethnobotany is considered as a department of Ethnobiology. Ethnobotany is a comprehensive approach which includes the reciprocal and vigorous thing of interaction of indigenous people with flora (**Schutles ,1962, Ford 1980**).

4.0 Objectives

- To enlist the herbals in Hingwadi and Hingwashtak churna
- To characterize macroscopic, microscopic and powder analysis of herbal and individual formulations
- To characterize the phytochemical screening of herbal and individual formulations
- To characterize the TLC, HPTLC and GCMS analysis of herbal and individual formulation
- To characterize the bio efficacies viz. antimicrobial and antioxidant efficacy of herbal and individual formation
- Generation of monograph for selected herbal formulations with reference to specific characteristics

5.0 Methodology

5.1 Collection of crude drugs

- Hingwashtak churna contains the crude drugs Sunthi, Marica, Pippali, Ajmoda, Sveta jirika, Krisna jirika, Hingu, Saindhava and same as Hingwadi churna contains crude drugs Hingu, Granthika, Dhanya, Jiraka, Vaca, Cavya, Agni, Patha, Sati, Vrksamla, Saindhava lavana, Sauvarcala lavana, Vida lavana, Sunthi, Marica, Pippali, Yava ksara, Svarjika Ksira, Dadima, Pathya, Puskara, Vetasamla, Hapusa, Ajaji, Ardraka rasa, Bijapura rasa. All these plant crude drugs required for the preparation of the standard formulation of Hingwashtak and Hingwadi churna will be collected from the local market of Jaipur. All the materials will be authenticated and used for formulation. Laboratory batches will be formulated and compared with marketed formulation. **(Deshmukh et al.,2018).**

5.2 Material & Methods

- Standardization and validation of Hingwashtak & Hingwadi churna will be performed using methods from The Ayurvedic Pharmacopoeia of India, part -1, volume II & edition first, Ministry of Health and Family Welfare Department of ISM & H,2007.
- The following steps will be involved-
- Foreign matter
- Macroscopic methods
- Microscopic methods
- Powdered microscopy

5.3 Foreign Matter (FM)

- plant material will be weighed and screen for foreign matter, which can be dust/debris other than plant material will be separated the weight of removing plant material and debris/ extra material weight will be calculated.
    - % Foreign material = Debris ÷ TPM × 100
    - e.g. – 2 ÷ 100 × 100 = 2% FM

- This FM % will be calculated in selected Hingwashtak & Hingvadi churna. Further, the limit of FM is checked as per The Ayurvedic Pharmacopoeia of India standards.

Macroscopic Method

every crude drug derived from the vegetable kingdom consists of a specific part of plant e.g., leaf, stem, root, wood, bark, fruit and so forth. morphological details of the respective component will be given by watching it with a necked eye or with the aid of a magnifying lens. In this trendy situations of the drug, length, shape, outer surface, Internal surface and so forth. sensory or organoleptic characters describe color, odour, taste, consistency and so forth **(API, 2007).**

Microscopic Methods

The microscopic examination of different element of the drug presents several diagnostic characters. Techniques of getting ready specimens of crude materials of vegetable drugs for microscopical research range, relying on the morphological companies of medicine will be tested and also on the natures of the fabric i.e., complete, cut or powdered. For analyzing leaves, herbs, flora, seeds, bark, and powdered beneath microscope, following strategies will be employed.

Powder microscopy of flower, leaves and cut materials

To examine entire leaves, herbs, and flowers, we will take pieces of leaf, herbs and flowers only calyx & corolla in test tube. And will add a solution of caustic alkali or nitric acid to the test tube and boil for 1-2 min and pour the contents into a porcelain dish, after that we will drain off the liquid and wash the material with water and leave for some time. After removing the pieces of the material from the water with spatula, we will put it on the slide and add few drops of solution of glycerol or chloral hydrate. And before examine we will crush the material with scalpel and cover this with cover slip. To examine cut leaves, herbs, and flowers. We will take several pieces in a test tube and employ the, same process as above mentioning.

To examine characters of the powder, we will take sufficient amount of powder in chloral hydrate solution on a slide and cover it. After that for a short time warm it over a low flame **(API,2007).**

Microscopy of fruits, seeds and powder

To microscopical examination of fruits and seeds, a sample or outer part of the seed or fruit will be taken and examined. To examine the outer coat, 2 or 4 seeds or fruit in caustic alkali solution will be placed in a test tube. Then boil for 1-2 min, if the outer coat is intensely pigmented, boil the samples for a longer time. After boiling, place the pieces on the slide. And will remove coated layers and test them by dipping them in the glycerol solution.

To examine the structure of the cells of the seed coat and the cells of the embryo will take a small amount of powder of the material on a slide in glycerol and cover it with a cover slip and examine. We will test also the presence of starch, fixed oil and mucilage in the seed with the help of iodine solution, chloral- hydrate solution, respectively **(API,2007).**

Microscopy of roots and rhizomes

We will cut transverse and longitudinal sections to physically examine the entire roots and rhizomes, and will soften the small pieces of roots without heating them in a solution of glycerol for 1-3 days depending on their hardness, then we will be able to straighten the soft roots in the right direction with the help of scalpel and then one by one with razor section will be cut. First cut into thick whole slices and then make thinner, smaller section. And then we will stain whole slices with phloroglucinol and concentrated hydrochloric acid or with safranin, after that we will examine the sample, with the help of a dissecting microscope. For microchemical testing, small and thin sections will be examined with a microscope. We will test also the presence of starch, insulin, lignified elements, fixed oil in the roots and rhizome. In chloral hydrate solution, we will prepare several samples of the powder on slides and then perform the mentioned standard tests for detection of starch, fixed oil, Insulin, lignified elements, tannins, mucilage etc. **(API.,2007)**

Physical parameters

In this parameter we will determine bulk density and tapped density for evaluating of physical characteristics of Hingwashtak and Hingwadi churna **(Deshmukh et al.,2018).**

Bulk density

Bulk density can be determined by transferring an accurately weighed amount of powder sample to the graduated cylinder with the aid of a funnel. Bulk density is the ratio of given mass of powder and its bulk volume.

Bulk density = w/v0 (g/ml)

where, w= mass of the powder

v0= untapped volume

Tapped density

The density can be determined as the ratio of mass of the powder to the tapped volume. It can be measured by transferring a known quantity of powder into a graduated cylinder a tapping it for a specific number of times.

Tap density = w/vf (g/ml)

Where, w= mass of the powder

Vf=tapped volume

Finger printing profiling by TLC, HPTLC and GC-MS

Powdered samples of Hingwashtak and Hingwadi churna (1.0 g each crude drugs) will be extracted separately with 15 mL methanol by sonication for 30 min at 45^{o}C. The process will be repeated two & three times to ensure complete extraction. The extracts obtained will be pooled and dried under reduced pressure. All the samples will be screened for foreign matter, macroscopy, microscopy thin layer

chromatography and other identification and characterization techniques for standardization of drugs **(Narasimhaji et al.,2019).**

TLC is a method in which a solute undergoes distribution between two phases, first is stationary phase acting through adsorption and second is mobile phase in the form of a liquid. TLC and HPTLC are methods normally implemented for the identity, the assay and the trying out for purity, stability, dissolution or content material uniformity of uncooked substances (natural and animal extracts, fermentation combos, drugs and excipients) and formulated products (prescription drugs, cosmetics, nutriments). These flexible and fee-effective strategies present the advantage of the simultaneous processing of standards and samples with versatile detection opportunities, together with a tremendous type of post-chromatographic derivatization reagents**.**

The idea of validation carried out to densitometric determinations on HPTLC indeed varies in keeping with the purpose of the evaluation and the stairs required for a formal validation have been very well investigated**.** Drastically for purity trying out, pharmaceutical dosage paperwork assay and herbals fingerprinting. The advances in chromatographic techniques made it viable to quantify the chemical elements in an aggregate with comparatively little easy-up using HPTLC **(Narasimhaji et al.,2019).** GC analysis will be carried out using method of khan and co-workers, 2017. Total run time will be calculated **(Khan et al.,2017)**.

The chemical evaluation includes quantitative chemical checks, qualitative chemical checks, chemical assays and instrumental analysis. The isolation, purification, and identity of lively components are chemical strategies of assessment. Qualitative chemical assessments consistof identification assessments for numerous phytoconstituents like alkaloid, tannins and so forth **(Khan et al.,2017)**.

Screening of Phytochemicals

The crude methanolic extracts of fruits, rhizome, leaves, seeds, exd. And bark will be tested for the presence of alkaloids, steroids, tannins, and saponins. The qualitative results will be expressed as (+) for the presence and (-) for the absence of phytochemicals.

Test for Alkaloids

Few mg (about 15mg) of each extract was separately stirred with 1% HCL (6mL) on a water bath for 5 min and filtered. These filtrates will be divided in 3 equal parts, for the applying different test such as Dragendorff' test, Meyer's test and Wagner's test to test alkaloids **(Joshi et al., 2013; Abdullahi et al.,2013).**

Steroids and terpenoids test

Salkowski test – We take crude extract will be separately shaken with chloroform followed by the addition of concentrated sulphuric acid along the side of the test tube, after this process reddish brown coloration interface indicates the presence of terpenoid **(Ayoola et al.,2008).**

Liebermann – Burchard test – We take each extract will shake with chloroform in a test tube then few drops of acetic anhydride will be added to the test tube and boil in a water bath and rapidly cool in ice water. Concentrated sulphuric acid will be added alongside of the test tube. After this process brown ring formation occurs at the junction of two layers and turning the upper layer to green shows the presence of steroids, and presence of triterpenoid shows deep red color **(Joshi et al., 2013**).

Tannins

we take crude drug extract will be separately stirred with distilled water and then filtered. Then will be added a few drops of 5% ferric chloride. Blue green and black coloration or precipitate will be taken as positive result for presence of tannins **(Banso and Adeyemo.,2006).**

Saponins

We take each of plant extracts will be separately shaken with distilled water in a test tube. Then the formation of frothing, which persists on warming in a water bath 5 min, then we checked presence of saponins **(Banso and Adeyemo.,2006).**

Assessment of Total Flavonoid (TFC)

Total flavonoid content- we will determine total phenolic content by various methods. Briefly, extract of crude drugs and standard will be placed in different test tubes and to each tube aluminum chloride, potassium acetate, methanol and distilled water will be added and mixed properly. A blank will be prepared in the same manner where distilled water will be used instead of the sample or standard, and the amount of aluminum chloride will be also replaced by distilled water. All tubes will be incubated at room temperature for 30 min. The absorbance will take at 415nm **(chang et al.,2002; Stankovic.,2011).**

Antibacterial and Antioxidant activities in Hingwadi and Hingwashtak Churna

Bacterial culture: - The standard pathogenic bacterial cultures will be taken from Biotechnology Lab of JECRC University, Jaipur, India.

Disc preparation for Antibacterial activities

The aqueous ethanol, methanol and acetone extracts will be prepared in their respective solvents and the sterile blotting paper disc will be soaked inside the diluted extract in such concentration that the amount of solution absorbed by every disc 5mg of every extract of natural preparations. The prepared disc will be dried in managed temperature to remove of extra of solvent and used for study **(Tambekar and Dahikar.,2011)**

Disc Diffusion Method for antibacterial activity

The reorient paper disc diffusion will be employed to determine the antibacterial action of both aqueous and organic extract of the natural arrangements **(NCCLS, 2002)**. In every test tube turbidity of inoculum will be matched with Mueller Hinton

broth turbidity standard. Inoculum will be spread over the nutrient agar plate using a sterile cotton swab in series to obtain consistent microbial growth. Then the organized antibacterial discs will be stored over the lawn and pressed slightly along with positive and negative control. Tetracycline or Streptomycin 10 mcg/disc will be used as positive control while disc soaked in sterile distilled water and various organic solvents and dried will be located on lawns as negative control. The plates will be incubated for 18 h at 37^{o} C. The antibacterial activity will be evaluated for 5 mg/disc and diameter of inhibition zones will be measured. Experiment will be carried out in triplicate and the averages diameter of zone of inhibition will be recorded. **(Tambekar and Dahikar.,2011)**

Antimicrobial Sensitivity Index (ASI) was calculated by following formula:

Antimicrobial Sensitivity Index for herbal preparation =

$$\frac{\text{Total zone of growth inhibition}}{\text{No. of antimicrobial agents tested} \times \text{No. of bacterial pathogens}}$$

6.0 Expected Outcomes of The Research

The Hingwadi and Hingwashtak churna is used for the remedy of numerous gastric sicknesses like acidity, indigestion, gastric ulcers, bloating, abdominal pain, joint disease, etc. Hingwashtak churna is likewise used as a treatment for Dysmenorrhea**.** The Hingwadi and Hingwashtak churna are worked on the calm's pitta and vata. They help to treat the symptoms of rheumatoid arthritis that means joint pain and swelling.

As they are of prime importance and possess a large no of ingredients which can be admix due to unavailability therefore standardization and validation is of utmost need. This validation can be useful at quality control measures to check the purity of the compound formulation and can be benefitted by local healers also. This validation can be used for preparation of monograph of the compound formulation and will scientifically validate the formulation. This will enhance the efficacy of the formulation and prevent the admixing of the other ingredients**.**

7.0 LIMITATIONS

The study takes in certain limitations-

1. We cannot test all phytochemicals that's why we will screen certain parameters for standardization methods as per the Ayurvedic pharmacopeia.

Year wise plan of work

1.	Literature review	Throughout the year
2.	Survey of traditional	0 to 2 months
3.	Collection and enlisting of crude drugs	2 – 8 months
4.	Experimentation for phytochemical and antimicrobial studies	8 – 20 months
5.	Fingerprinting profiling by HPTLC, TLC, GC-MS	20 – 27months
6.	Statistical analysis	27 –29months
7.	Result and interpretation	29 – 31 months
8.	Preparation of thesis	31 – 36 months

Year wise plan

Activities/Months	0 – 2	2 – 8	8 – 20	20 – 27	27 –29	29 – 31	31 – 36
Literature review							
Survey of traditional healers							
Collection and enlisting of crude drugs							
Experimentation for phytochemical and antibacterial studies							
Fingerprinting profiling by HPTLC, TLC, GC-MS							
Statistical analysis							
Result and interpretation							
Preparation of thesis							

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